Targeted RNA studies offer new opportunities to reduce uncertainty and increase diagnostic yield

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RNA analysis has emerged as a valuable tool for providing lines of evidence about the classification of putative splicing variants. Recently, some clinical laboratories have started offering targeted RNA analysis to clarify the pathogenicity of variants identified by DNA testing.

Here, we reviewed the outcome of targeted RNA studies between January 2018 and May 2025 for exome and neurology panels. Criteria for RNA studies include variants in characterized genes with clinical relevance to the proband, predicted splicing impact by *in silico* models, sufficient gene expression in blood, and an established loss-of-function mechanism of disease. Variants were either identified through testing at our laboratory (prospective) or by request for variants identified at external laboratories (retrospective). We examined the origin of cases, frequency over time, and the diagnostic impact of RNA studies.

RNA studies were performed for 50 unique variants in 44 genes. *FOXP1, IFT140, ITGB2, LZTR1, RMND1,* and *SLC20A2* had more than one variant. 86% (n=43) of variants were intronic, 10% (n=5) were missense, 2% (n=1) nonsense, and 2% (n=1) silent. Most variants (90%; n=45) started as VUS, 8% (n=4) were likely pathogenic (LP), and 2% (n=1) were pathogenic (P).

RNA studies resulted in a reclassification of 52% (n=26), and 88% (n=23/26) of reclassifications resulted in P/LP finding. Overall, the diagnostic rate in this cohort increased from 10% (n=5) to 56% (n=28). There was a 56% relative decrease in VUS from 45 to 20, with most VUS upgraded to LP or P. In two instances, RNA studies resulted in a VUS downgrade to likely benign.

There were more external cases (n=33) compared to internal cases (n=17). An influx of external cases were seen in 2024 when an exome-paired RNA studies offering was launched. There were no significant differences in VUS reclassification between internally detected variants and externally referred cases (p=0.7575 for VUS upgrades and p=1.0 for VUS downgrades), suggesting RNA studies are equally as useful proactively compared to retrospectively selecting variants.

RNA studies reduce the number of VUS and increase the diagnostic yield. Targeted RNA studies are a practical way to generate the evidence required for ES VUS resolution. The availability of RNA studies to clarify VUS on exome and genome sequencing at the time of testing will further increase the clinical utility of these tests.

Learning objective: Examine the outcomes of RNA studies for variant reclassification in a cohort of prospective exome/neurology panel cases compared to externally referred cases